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Block of the delayed rectifier current (I_K) by the 5-HT_3 antagonists ondansetron and granisetron in feline ventricular myocytes

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1 We investigated the effects of two 5-HT_3 antagonists, ondansetron and granisetron, on the action potential duration (APD) and the delayed rectifier current (I_K) of feline isolated ventricular myocytes. Whole-cell current and action potential recordings were performed at 37°C with the patch clamp technique.

2 Ondansetron and granisetron blocked I_K with a K_D of 1.7 ± 1.0 and 4.3 ± 1.7 μM, respectively. At a higher concentration (30 μM), both drugs blocked the inward rectifier (I_{K1}).

3 The block of I_K was dependent on channel activation. Both drugs slowed the decay of I_K tail currents and produced a crossover with the pre-drug current trace. These results are consistent with block and unblock from the open state of the channel.

4 Granisetron showed an intrinsic voltage-dependence as the block increased with depolarization. The equivalent voltage-dependency of block (δ) was 0.10 ± 0.04, suggesting that granisetron blocks from the intracellular side at a binding site located 10% across the transmembrane electrical field.

5 Ondansetron (1 μM) and granisetron (3 μM) prolonged APD by about 30% at 0.5 Hz. The prolongation of APD by ondansetron was abolished at faster frequencies (3 Hz) showing reverse rate dependence.

6 In conclusion, the 5-HT_3 antagonists, ondansetron and granisetron, are open state blockers of the ventricular delayed rectifier and show a clear class III action.

Keywords: Myocardium; potassium channels; delayed rectifier; inward rectifier; action potential duration; class III effect

Introduction

Ondansetron and granisetron are two 5-HT_3 receptor antagonists that are used clinically to prevent nausea and vomiting induced by chemotherapy (Aapro, 1991). In addition, preclinical studies suggest that this class of compounds might have clinically useful anxiolytic and antipsychotic actions (Costall *et al.*, 1990). Several studies have also shown that 5-HT_3 antagonists of different chemical structure have significant electrophysiological effects in cardiac muscle. Prolongation of the QT_c electrocardiographic interval in anaesthetized laboratory animals has been reported with tropisetron (Hof *et al.*, 1993), ondansetron and zatosetron (Williams *et al.*, 1991). The effects of tropisetron (ICS 205–930), an indole, have further been examined in cellular electrophysiological studies in guinea-pig papillary muscle, where tropisetron prolonged action potential duration at 30% and 70% of repolarization (APD_{30} and APD_{70}) (Scholtysik, 1987). However, in sheep Purkinje fibres, tropisetron did not have any significant effect on APD_{90} and did shorten APD_{50} (Williams *et al.*, 1985). In a study in guinea-pig ventricular myocytes at concentrations that were high compared to the therapeutic range, tropisetron showed both class I and class III antiarrhythmic actions and blocked potassium, sodium, and calcium currents (Scholtysik *et al.*, 1988). Although tropisetron has been reported to have antiarrhythmic effects in several animal models (Williams *et al.*, 1985; Coker *et al.*, 1986; Hof *et al.*, 1993), a clinical study found limited antiarrhythmic activity in patients with ventricular arrhythmias (Morganroth & McGinn, 1988), while a second clinical report suggested that granisetron might produce serious disturbances of cardiac rhythm, including ventricular arrhythmias and cardiac arrest (Ballard *et al.*, 1992).

In this study, we report that both ondansetron, a carbazole, and granisetron, an indazole, prolonged APD in cat ventricular myocytes. In voltage clamp studies, we examined the effects of both compounds on the delayed rectifier (I_K) and inward rectifier (I_{K1}) potassium currents, as block of these channels is known to delay repolarization. The aim of this study was, first, to investigate the mechanism by which ondansetron and granisetron prolong APD, and, second, to characterize the mechanism of this block. These results could be useful in developing new class III antiarrhythmic agents and in understanding the mechanisms of block of I_K channels.

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Methods

Cell preparation

Ventricular myocytes were isolated from the myocardium of pentobarbitone-anaesthetized cats weighing 2–4 kg, according to a modification of the method described by Silver *et al.* (1983). Briefly, the hearts were isolated and perfused on a Langendorf apparatus with nominally Ca^{2+}-free Krebs-Henseleit (KH) solution at 37°C for 4 min. The KH contained (in mM): NaCl 130, KCl 4.8, $MgSO_4$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 25, dextrose 12.5. The pH was 7.35–7.40 when equilibrated with 95% O_2:5% CO_2. The hearts were then enzymatically digested for 12–15 min with a nominally Ca^{2+}-free KH containing 0.7 mg ml^{-1} of type II collagenase (197/u mg^{-1}; Worthington, Freehold, NJ, U.S.A.). The ventricular tissue was dissected from the atria, minced, and then passed through a 200 μm pore nylon mesh. The filtrate was centrifuged at 720 r.p.m. for 1–2 min. Afterwards, the pellet was re-suspended in Ca^{2+}-free KH. The process was repeated two more times and the pellet then re-suspended in KH containing 2% bovine serum albumin (BSA) and 100 μM Ca^{2+}. The cells were allowed to equilibrate for 5 min at 37°C under a 95% O_2:5% CO_2 gas curtain. The Ca^{2+} concentration was raised after 5 min to 0.5 mM. The ventricular myocytes were plated in 35 mm Petri dishes and stored under controlled

humidified 95% O_2:5% CO_2 atmosphere. The myocytes were used within 8 h of isolation.

Current and voltage recordings

Potassium currents were recorded with the patch-clamp technique in the whole-cell configuration. For recording of transmembrane action potentials the voltage clamp amplifier was switched to the current clamp mode. Action potentials were elicited by 10–15 ms long depolarizing current pulses delivered through the microelectrode. The patch electrodes were made of Kimax-51, a borosilicate glass from Kimble (Toledo, OH, U.S.A.), of 1.5 mm outside diameter. They were pulled on a Narashige vertical puller (Narashige PP-83, Tokyo, Japan) by the 2-stage method. The pipette solution contained (in mM): K-aspartate 145, NaCl 5, $MgCl_2$ 1, EGTA 10, HEPES 5, ATP·Mg 5, phosphocreatine-Na_2 5, pH ajusted to 7.2 with KOH. Electrodes had a resistance of 3–5 MΩ when measured in the bath solution. The external medium was a modified Tyrode solution of the following composition (in mM): NaCl 138, KCl 4, $CaCl_2$ 2, $MgCl_2$ 0.5, $NaHCO_3$ 24, dextrose 5.5. The solution was aerated with 95% O_2:5% CO_2 and the pH was 7.35–7.40. Recordings were performed with a List EPC-7 amplifier (List-Medical, Darmstadt, Germany) coupled either to a Basic-Fastlab (Indec Systems; Sunnyvale, CA, U.S.A.) or pClamp (Axon Instruments, Foster City, CA, U.S.A.) data acquisition and analysis software. Signals were low-pass filtered with a corner frequency of 3 kHz. The reference zero potential was adjusted in the bath solution before forming the seal. The pipette-cell membrane seals showed a resistance greater than 2 GΩ. Capacitance was optimally compensated. Series resistance was 3.54 ± 0.31 MΩ (14 cells), producing for a 100 pA current a 0.35 mV uncertainty on the potential. Data were sampled at 0.5–1.0 kHz, except for the I_{K1} experiments (2 kHz) and the studies of recovery from block (4 kHz).

Data analysis

The delayed rectifier current was analysed from outward tail currents elicited upon repolarization to the holding potential (− 45 mV) from various conditioning potentials (− 45 mV to + 45 mV). Two pulse durations were used, 250 ms and 2500 ms, the former being more relevant to the duration of an action potential and the latter allowing a more complete activation. Concentration-response curves were constructed from the percentages of unblocked tail currents. The concentration-response data were curve-fitted according to the relationship:

$$Y = Y_{max}\text{-}[(Y_{max} - Y_{min})/(1 + (K_D/A)^H)] \quad (1)$$

where Y_{max} and Y_{min} are the theoretical maximum and minimum values of the function. A is the concentration of antagonist (ondansetron or granisetron), K_D the dissociation constant, and H the Hill coefficient. The parameter Y_{max} was set at 100%; Y_{min}, K_D and H were left free during computation.

Activation curves were analysed with a Boltzmann distribution according to the following equation:

$$I = I_{max}/[1 + \exp(V_{1/2}\text{-}V)/k] \quad (2)$$

where I is the recorded tail amplitude and V is the test potential. The potential, V, is the independent variable. The maximum current (I_{max}), the voltage of half activation ($V_{1/2}$), and the slope factor (k) were left free during computation.

The voltage-dependence of block was analysed with a Boltzmann distribution, according to the following equation (Woodhull, 1973):

$$1\text{-}Y = A/(A + (K_D \cdot \exp(-z\delta V/KT)) \quad (3)$$

where K_D is the theoretical dissociation constant at 0 mV, z the charge number, K the Boltzmann constant, T the absolute temperature, and δ the fractional equivalent voltage-dependency. Other variables are as above. K_D and δ were the computed parameters, V was the independent variable.

A non-linear least squares iterative algorithm based on the Gauss-Newton iterative solution method was used to estimate the variables of the equations. The programme was written by Dr K. Goldberg at Wyeth-Ayerst Research and was run on a HP-9836 computer. Uncertainty on computed parameters was assessed from their asymptotic variance. The decay of tail currents was fitted to exponential functions using a nonlinear iterative procedure (Axon Instruments pCLAMP clampfit programme).

Student's t test (paired and unpaired) was used for statistical comparisons. The level of significance was set at $P < 0.05$. Values are expressed as mean ± s.e.mean.

Determination of pK_a and partition coefficients

The values of pK_a were determined by Mr D. Herold (Ancillary Services, Wyeth-Ayerst Research) using a Sirius PCA 101 titrator (Sirius Analytical Instruments Ltd., East Sussex, UK). Octanol/phosphate buffer (pH = 7.4) partition coefficients were determined by Mr J.M. Kulishoff, Jr., (Ancillary Services, Wyeth-Ayerst Research) by high performance liquid chromatography (h.p.l.c.) analysis.

Drugs

Hydrochloride salts of ondansetron (Glaxo Group Research Ltd., Greenford, Middlesex, UK) and granisetron (Smith-Kline Beecham Pharmaceuticals, Betchworth, Surrey, UK) were dissolved in distilled water. Experiments were performed in the presence of nisoldipine (300 nM), which was predissolved in 70% ethanol for a final vehicle concentration of 0.1%. Nisoldipine was a gift from Miles Inc. (West Haven, CT, U.S.A.). All experiments were conducted at 37°C.

Animal care

Animals were cared for and used in accordance with the Animal Welfare Act as amended. Protocols were examined and approved by the Institutional Animal Care Use Committee (IACUC).

Results

Prolongation of action potential duration

Control action potential duration (APD) of cat ventricular myocytes during pacing at 0.5 Hz at 37°C was 456 ± 34 ms (9 cells). Superfusion with ondansetron (1 μM) and granisetron (3 μM) produced a marked lengthening of APD_{90} (Figure 1), from 479 ± 23 to 625 ± 44 ms with ondansetron ($n = 5$) and from 427 ± 71 to 575 ± 117 ms with granisetron ($n = 4$). With ondansetron, APD_{90} returned to pre-drug values after 5 min in drug-free Tyrode solution. With granisetron, the effect was not totally reversible even after prolonged washout: after 15 min, APD_{90} was 478 ± 90 ms. Neither ondansetron nor granisetron affected action potentional amplitude or resting membrane potential. Resting potential values for the ondansetron and granisetron groups were − 78 ± 2 and − 73 ± 3 mV before drug exposure, − 78 ± 3 and − 74 ± 5 mV in presence of drug, and − 78 ± 3 and − 74 ± 5 mV after washout, respectively. Amplitude was 130 ± 4 and 127 ± 3 mV in pre-drug, 129 ± 4 and 123 ± 5 mV in presence of drug and 128 ± 4 and 124 ± 6 mV in washout, respectively. The results of these studies show that ondansetron and granisetron have a clear class III effect.

Block of the delayed rectifier (I_K) current

The mechanism of action of ondansetron and granisetron was examined by studying their effects on the delayed

rectifier current. Under control conditions (Figure 2a and c), depolarization steps from −45 to +45 mV elicited, immediately after the capacitive transient, rapidly decaying transient outward currents (I_{to}), which were followed by slowly developing outward currents. Upon repolarization to −45 mV, each trace was followed by a slowly decaying outward current tail. These tail currents have been shown to represent the deactivation of the delayed rectifier current (Noble & Tsien, 1969), and a measurement of their peak amplitude in reference to holding current provides an estimate of the I_K current activated during the depolarizing step. Conditions were adjusted to minimize inward currents that are normally activated by such depolarizing steps. The fast inward Na^+ current (I_{Na}) was inactivated by holding the cell at a depolarized potential (−45 mV), while the L-type Ca^{2+} current was almost totally blocked by 300 nM nisoldipine in Tyrode solution. After a 6 min exposure to 10 μM ondansetron (Figure 2d) and a 5 min exposure to 10 μM granisetron (Figure 2b), the amplitude of tail currents was significantly reduced. After granisetron, the holding current became less positive by about 70 pA. In 4 similar studies at 10 μM, the holding current was reduced by 52 ± 12 pA. The effects on tail current amplitude and holding current were reversed upon drug washout (not shown). Ondansetron did not affect holding current. Despite the profound block of tail currents, the effect of both compounds on the time-dependent current that developed during the depolarizing step was variable. Previous studies have shown that this current is variable in size and in drug sensitivity among myocytes (Follmer & Colatsky, 1990; Spinelli *et al.*, 1993).

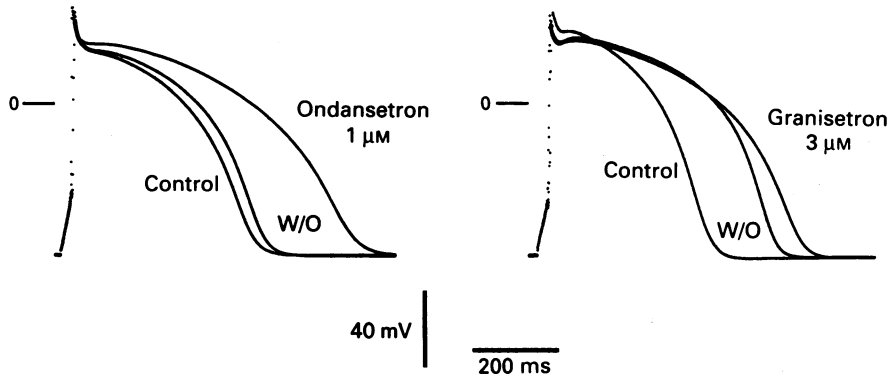

Figure 1 Effects of ondansetron and granisetron on the transmembrane action potential of cat ventricular myocytes. Each tracing represents an average of 10 action potentials obtained at steady-state from two different cells. Temperature: 37°C; stimulation rate: 0.5 Hz.

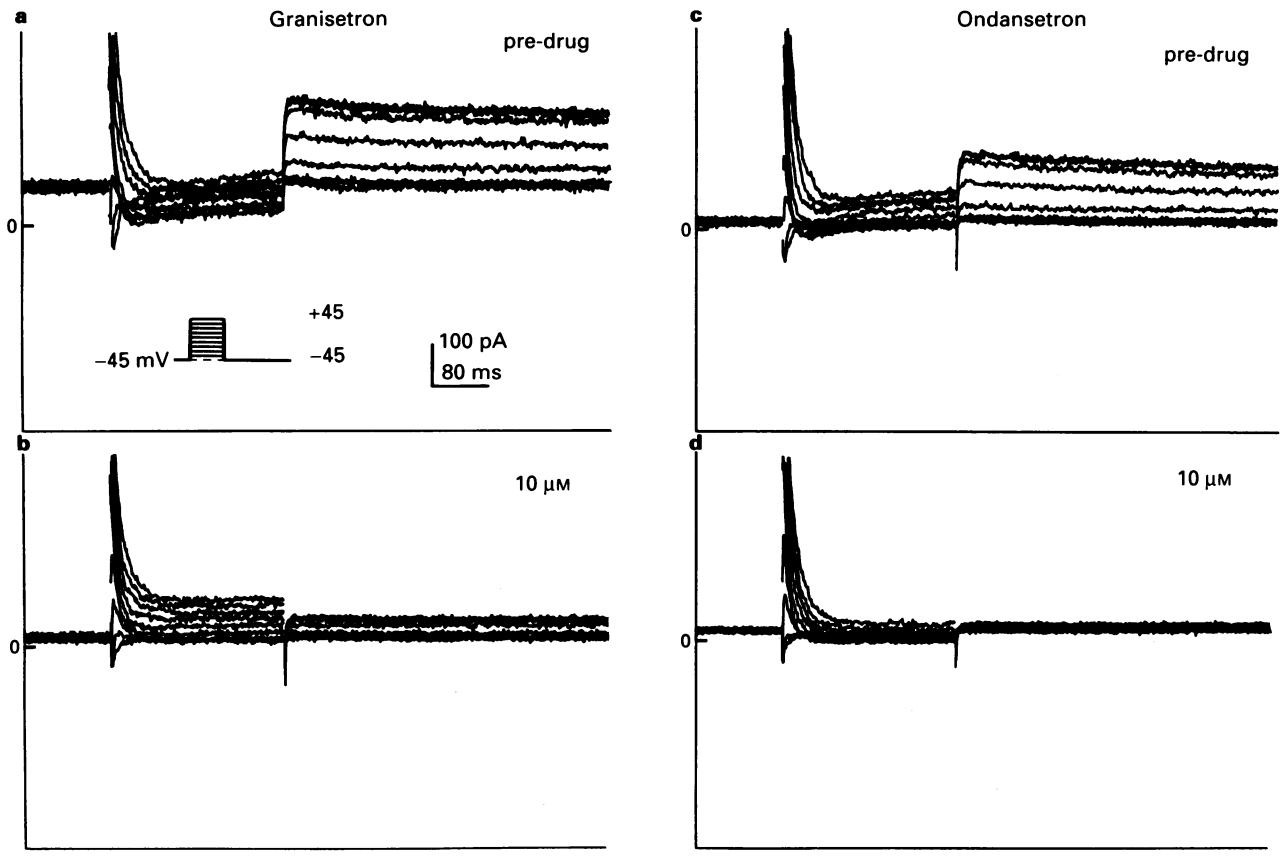

Figure 2 Effect of granisetron and ondansetron on the delayed rectifier tail currents in two different myocytes. The voltage protocol is shown in (a); step duration: 250 ms. Panels (a) and (c), pre-drug condition. Panels (b) and (d), after a 5 to 6 min exposure to 10 μM of either drug. Nisoldipine (300 nM) was present to block $I_{Ca\text{-}L}$. All experiments performed at 37°C.

For these reasons, in this study we only describe the effects of ondansetron and granisetron on I_K tail currents.

Activation parameters

The drug effect on the voltage-dependent activation of the delayed rectifier was studied from the amplitude of tail currents at − 45 mV following step depolarizations to test potentials between − 35 and + 45 mV. Figure 3b and d show the effects of ondansetron (1 μM, $n = 5$) and granisetron (3 μM, $n = 4$) on the activation curves obtained with 2500 ms-long test pulses. These drug concentrations were selected because they produced similar prolongation of APD and similar inhibition of tail currents (see below). Previous studies had also shown that maximum tail current amplitude did not further increase after 5000 ms pulses, showing that a 2500 ms pulse produces full activation (Spinelli *et al.*, 1993). With both compounds, the voltage-dependent activation of tail currents was well fitted by the Boltzmann function. Maximum pre-drug current at maximum activation (I_{max}) was 155 ± 3 and 202 ± 3 pA for the cells treated with granisetron and ondansetron, respectively. Despite the different maximum currents, the pre-drug parameters of activation were similar for both groups. Ondansetron (1 μM, Figure 3b) reduced the current at maximum activation to 63 ± 2% of the pre-drug value. The drug shifted $V_{1/2}$ by 2.7 ± 0.9 mV in the negative direction (from − 10.1 ± 0.7 to − 12.8 ± 1.2 mV) without affecting the slope factor (k: from 8.5 ± 0.6 to 9.1 ± 1.0 mV). After a 10 min washout, $V_{1/2}$ returned to the pre-drug value (− 10.4 ± 1.0 mV) and I_{max} recovered to 90 ± 2% of the pre-drug value. Granisetron (3 μM; Figure 3d) had similar effects, reducing I_{max} to 57 ± 2% and shifting $V_{1/2}$ by 6.4 ± 1.1 mV (from − 8.3 ± 0.5 to − 14.7 ± 1.9 mV), without altering the slope factor (k: from 7.7 ± 0.6 to 6.9 ± 1.6 mV). The effect of granisetron was reversed to a lesser degree even after prolonged washout (10–15 min): I_{max} recovered to 77 ± 1.6% of pre-drug value and $V_{1/2}$ returned to − 10.1 ± 0.9 mV.

The activation of I_K tail currents in the same groups of cells was also studied with 250 ms-long test pulses, a duration more relevant to the duration of action potentials. The resulting tail currents were, at full activation, 10 to 20 pA smaller in amplitude than those observed after 2500 ms-long depolarization steps (Figure 3a and c). Again, although pre-drug I_{max} were different between the two groups (146 ± 3 pA for ondansetron and 187 ± 4 pA for granisetron), the activation parameters were similar. Both compounds reduced I_{max} to a similar extent and shifted the activation curve $V_{1/2}$ in the negative direction by 3.1 ± 1.0 mV for ondansetron and by 5.3 ± 0.7 mV for granisetron (from 4.2 ± 0.9 to 1.1 ± 0.5 mV and from 4.7 ± 0.9 to − 0.6 ± 0.6 mV, respectively) without affecting the slope factors.

Comparison of activation curves obtained after 2500 ms-long (Figure 3b,d) and 250 ms-long steps (Figure 3a,c) reveals that I_{max} values at 250 ms are only slightly lower, both in controls and in presence of drugs. The percentage of block at each voltage is similar at both durations. These results suggest that: (1) the currents activated with the shorter step protocol (250 ms) were close to full activation, and that: (2) the amount of block did not vary between 250 ms and 2500 ms. However, the activation curves obtained with the longer pulse protocol showed a significant shift to the left indicating that more current was activated at more negative test potentials. This observation is quantified by a 12 mV shift of $V_{1/2}$ values in the negative direction with the 2500 ms protocol.

Concentration-response relationships

The concentration-response curves for block of the I_K tail current are shown in Figure 4. The data are presented as percentage of pre-drug amplitude after 2500 ms depolariza-

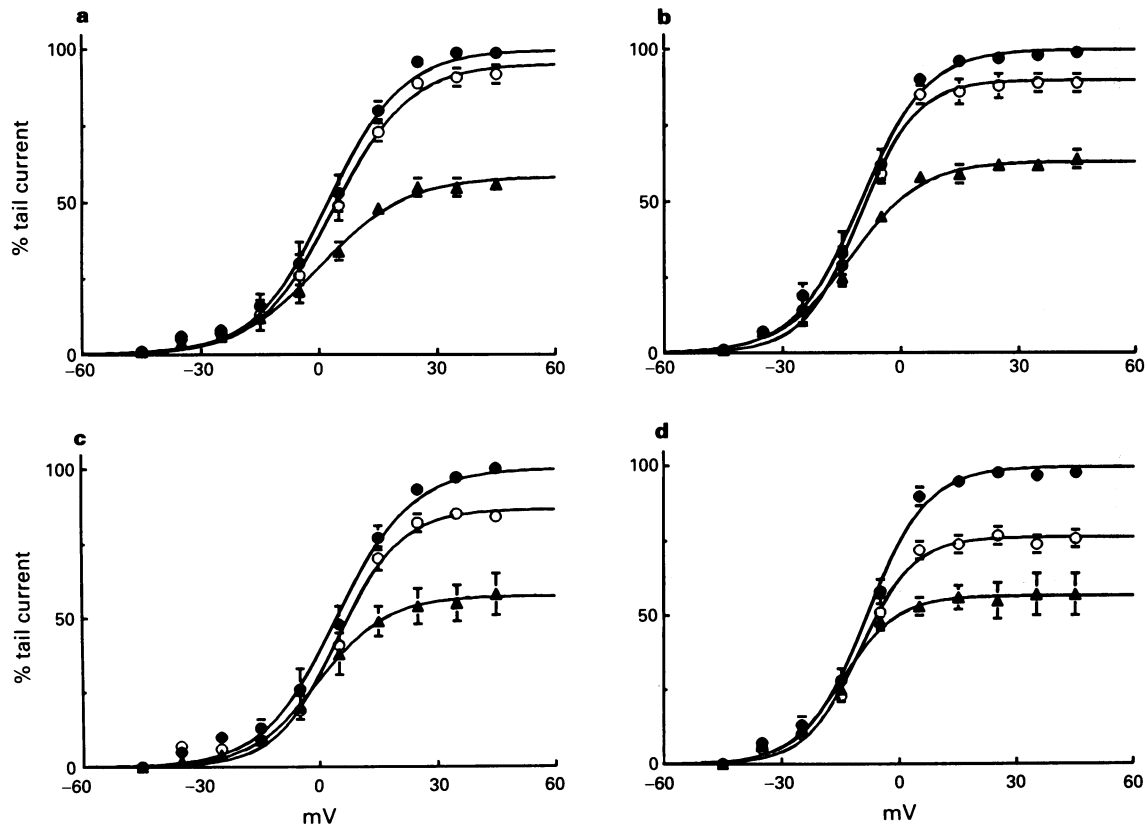

Figure 3 Effects of ondansetron (1 μM, a,b) and granisetron (3 μM, c,d) on the voltage-dependent activation of I_K tail currents: (●) pre-drug controls; (▲) in presence of drug; (○) washout. Two conditioning pulse durations were used for each cell: 250 ms (a,c) and 2500 ms (b,d). All curves normalized to maximum activation (I_{max}) in pre-drug condition.

tions to + 35 mV. The potential and duration of the test pulse were chosen to insure that tail current amplitude reached steady state (see above). Drug concentrations were applied in a cumulative way and each concentration was superfused for 5 to 8 min before the effect on tail currents was measured; this time interval was sufficient to reach a steady-state effect. The data were curve-fitted according to the general equation for receptor occupancy (eq. 1, see Methods). The dissociation constants were estimated to be 1.7 ± 1.0 μM for ondansetron ($n = 13$) and 4.3 ± 1.7 μM ($n = 8$) for granisetron. The Hill coefficients were not significantly different, and were 0.64 ± 0.33 and 0.78 ± 0.37 for ondansetron and granisetron, respectively. The effects of both compounds were not totally reversed by a 10–15 min washout for ondansetron and a 15–25 min washout for granisetron. In summary, ondansetron and granisetron showed good efficacy as blockers of I_K tail currents. Ondansetron was about 2.5 times more potent than granisetron, with both drugs showing similar Hill coefficients.

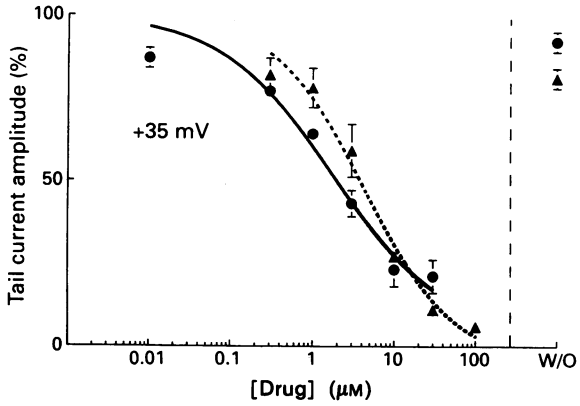

Figure 4 Concentration-response relationships were obtained from the amplitude of tail currents following a 2500 ms-long pulse at + 35 mV. The block of I_K is reversible upon washout (W/O). Each point represents an average of 3 to 9 cells. Ondansetron (●) 13 cells; granisetron (▲), 8 cells.

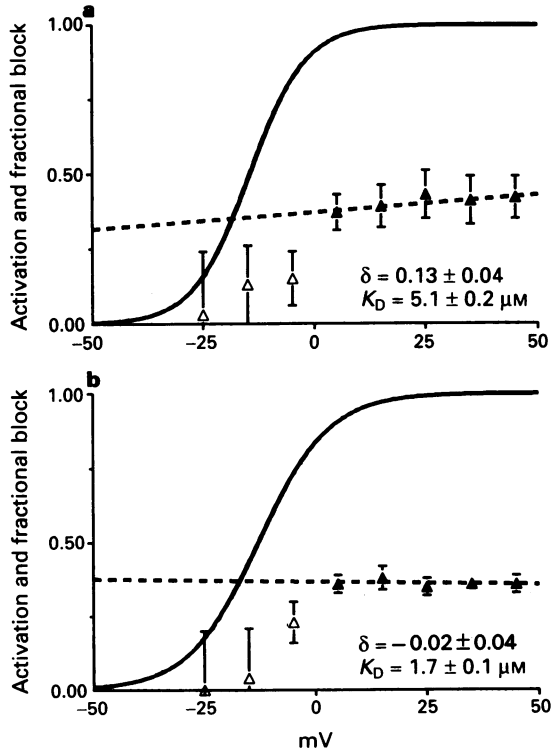

Figure 5 Normalized pre-drug activation curve (solid line) and fractional block (broken line, eq. 3) by granisetron (3 μM; a) and ondansetron (1 μM; b) illustrating the dependence of the block on the activation process and voltage. Only granisetron showed an intrinsic voltage dependence. K_D: dissociation constant at 0 mV; δ: equivalent voltage-dependency, as calculated from eq. 3.

Activation-dependence of block

Analysis of the activation curves showed that little block is observed at voltages more negative than − 5 mV and that block increases with stronger depolarizations, suggesting that the block might be dependent on channel activation. To analyse this phenomenon, the amplitude of the tail current at each potential in the presence of drug is normalized to the amplitude obtained in pre-drug conditions ($I_{drug}/I_{control}$) (Figure 5a and b). With both drugs, the initial degree of block (shown by unfilled symbols) increased steeply in the voltage range of channel activation, until it reached a plateau level at potentials more positive than + 5 mV (shown by filled symbols), when full activation was obtained. A similar profile of block was observed at all drug concentrations (not shown) and it is consistent with the view that ondansetron and granisetron blocked the channel while in the open state.

With granisetron, the degree of block showed a further limited but clear increase between + 5 and + 45 mV, a voltage range where all channels are in the open state. Although the increase of block exhibited a shallow slope, it was observed at all concentrations and it could not be explained by channel gating as it was observed at voltages and at a time (2500 ms) where the activation process had already reached a maximum. Thus, these results were suggestive of a limited, intrinsic, voltage-dependent component in the block by granisetron. Considering that granisetron is a weak base ($pK_a = 9.6$) and thereby most of its molecules will bear a positive charge at physiological pH, it is possible that the voltage-dependency might result from the effect of the transmembrane electric field on the positively charged granisetron molecule. To quantify the voltage sensitivity of the block, the fractional block at full activation was fitted using a Boltzmann distribution function (Woodhull, 1973). The data obtained with 3 μM granisetron (Figure 5a) yielded an equivalent voltage-dependency (δ) of about 0.13, which means that the positively charged molecule is subject to 13% of the transmembrane electric field at its site of action. The computed dissociation constant for 0 mV was 5.1 ± 0.2 μM.

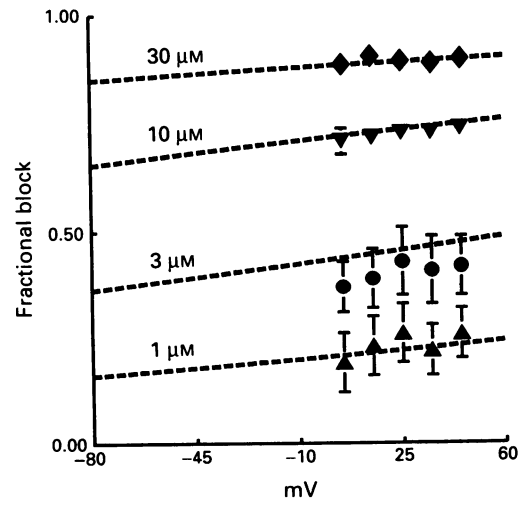

Figure 6 Voltage- and concentration-dependence of I_K block by granisetron showing the best simultaneous fit using eq. 3 for the indicated concentrations. Compared parameters: equivalent voltage-dependency, δ = 0.10 ± 0.04, and dissociation constant at 0 mV, K_D = 3.9 ± 0.7 μM. The lines represent the constrained fit.

This voltage-dependence was consistently observed at various concentrations. The best simultaneous fit of all the data sets obtained is shown in Figure 6. It yielded an equivalent voltage-dependency of 0.10 ± 0.04 and a dissociation constant for 0 mV of 3.9 ± 0.7 μM, in fair agreement with the previously calculated K_D.

Ondansetron is a much weaker base than granisetron, with a pK_a of 7.4, and thus at physiological pH only 50% of the molecules will be positively charged. Over the entire range of concentrations studied, we did not observe any indication of voltage dependency in its blocking action. The results with 1 μM are shown in Figure 5b.

Recovery from block

Analysis of the tail currents showed that the time course of decay was considerably slowed in the presence of drug. This could result from channels that unblock after repolarization and thus contribute to the conductance of outward current before they completely deactivate. Kinetic analysis of this phenomenon is difficult in cat myocytes under normal conditions because the decay of control tail currents at depolarized voltages (e.g. − 40 mV) is very slow and does not allow a reliable estimate of the deactivation time constant. To accelerate the decay of the tail current, 0.2 mM $CdCl_2$ was added to the Tyrode solution. Addition of Cd^{2+} has been shown to speed the decay of I_K tail currents (Follmer *et al.*, 1992b) without affecting their sensitivity to blockers of the delayed rectifier (Colatsky *et al.*, 1994).

The tail current decay was usually well fitted by a single exponential function, but some cells (6 out of 16) showed a second smaller and slower exponential component. In these cases, only the first component describing the fast initial decay of I_K was analysed. The smaller currents of the late phase are of undetermined nature and could include a second component of I_K, or a contamination by electrogenic transporters or other currents. Figure 7a shows the effect of 1 μM ondansetron on the decay of a tail current elicited on repolarization to − 60 mV following a 1000 ms conditioning step at + 40 mV. In the presence of drug, τ increased from 444 ms to 653 ms and the tail current in the presence of drug crossed the control trace after 600 ms. A rounding and delay of the peak of the tail (of about 20 ms) also occurred. In 7 myocytes, τ increased from 242 ± 50 ms to 337 ± 76 ms (+ 39%). These changes of time course are consistent with the view that open channels can release drug upon repolarization and then become available to conduct outward current before they deactivate. Similar results were obtained with granisetron (3 μM) in 9 cells, where τ increased at − 60 mV, from 256 ± 50 ms to 327 ± 52 ms (+ 28%). A significant delay of the peak current (> 8 ms) was observed with both drugs in 9 out of 16 cells and averaged 21 ± 3 ms. In the remaining cells, the delay was too small to be clearly measured against the background noise of the current trace. Figure 7b and c show that the deactivation of tail currents was voltage-dependent. Both drugs slowed the decay of the tail currents and the slowing, in percentage, seemed to decrease with depolarization.

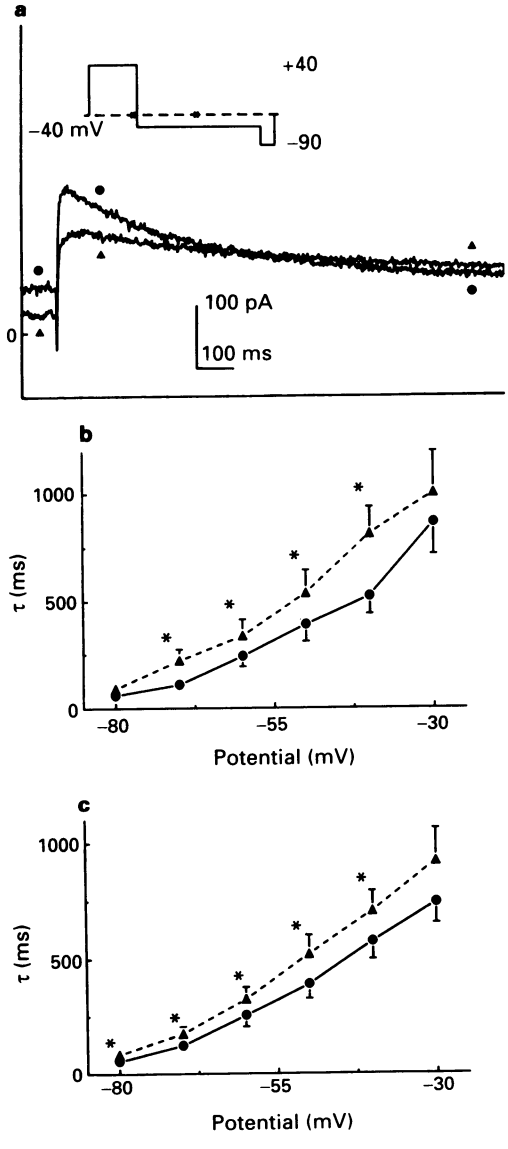

Figure 7 (a) Effects of ondansetron (1 μM) on the rate of decay of I_K tail current. Both ondansetron and granisetron (not shown) consistently induced a slowing of the decay and a crossover with the pre-drug current trace. Control (●); ondansetron (▲). In the example, τ increased from 444 to 653 ms. Tail currents recorded at − 60 mV, after a 1000 ms conditioning pulse. The time window displayed is indicated by the two stars in the protocol. (b) and (c) Time constants of tail decay as a function of voltage. Ondansetron (b) (7 cells) and granisetron (c) (9 cells); $^*P < 0.05$ (paired Student's *t* test).

Frequency dependence of APD prolongation

The prolongation of APD was studied as a function of the frequency of stimulation. Frequency was increased from 0.3–0.5 to 3 Hz and then returned to 0.3–0.5 Hz to verify the stability of the preparation. Figure 8 shows the results from four cells. Ondansetron was chosen because its action in blocking I_K tails did not show intrinsic voltage-dependence and thus the results can be interpreted solely in terms of kinetics of block. Prolongation of APD by ondansetron (1 and 2 μM) was abolished at 3 Hz. Such a decrease of efficacy in prolonging APD by other class III agents has been termed 'reverse use dependence' (Hondeghem & Snyders, 1990).

Effects on the inward rectifier (I_{K1}) current

Since the inward rectifier, I_{K1}, is known to contribute to the late phase of repolarization (Giles & Imaizumi, 1988), we examined whether ondansetron and granisetron block I_{K1}. Ondansetron (1 μM) had no detectable effects on holding current at − 80 mV, reversal potential, or whole-cell conductance from − 100 to − 25 mV, a voltage-range where I_{K1} is predominant. The block of I_K is apparent at more positive voltages (Figure 9). Similar results were obtained in 3 more cells. Similarly, granisetron at 3 μM, an equipotent concentration for blocking the delayed rectifier, did not have any significant effect (not shown). These results are consistent with the lack of effect on the resting potential observed with both compounds at these concentrations in the current-clamp studies. At a higher concentration, ondansetron (10 μM) did not have any detectable effect either ($n = 3$, not shown). However, both ondansetron and granisetron at 30 μM produced a significant block of I_{K1} as shown by the decrease of

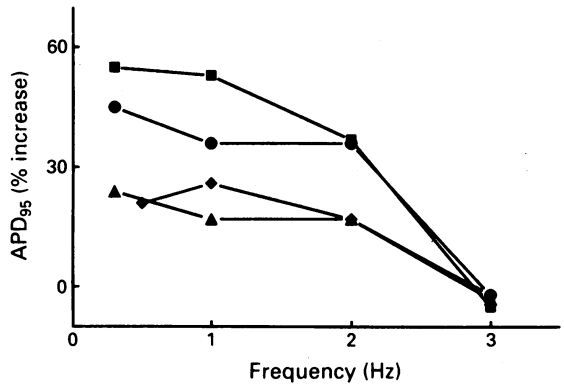

Figure 8 Frequency-dependency of APD prolongation by ondansetron (▲; ◆: 1 μM; ●, ■: 2 μM) in four myocytes. Experimental points represent an average of 6 to 15 action potentials recorded at steady-state.

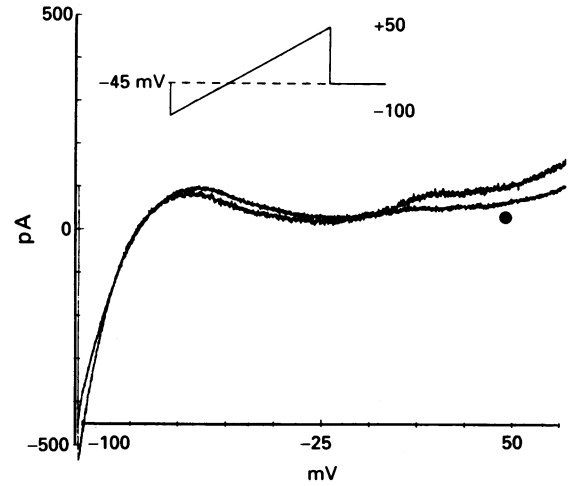

Figure 9 Effects of ondansetron (1 μM) on the quasi steady state current-voltage relationship in one myocyte. Ondansetron had little effect from − 100 to − 25 mV, a voltage range where the inward rectifier current, I_{K1}, is predominant. (●) current in presence of drug; voltage ramp, 3.34 mV s^{-1}.

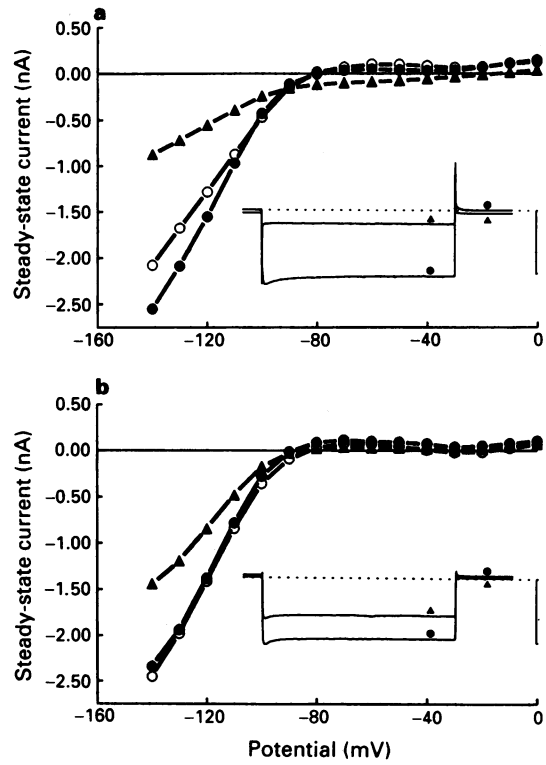

Figure 10 Effects of granisetron (a) and ondansetron (b) on I_{K1} in two different myocytes: (●) pre-drug; (▲) 30 μM drug; (○) washout. Current amplitude was measured at the end of 500 ms-long test pulse stepped from a holding potential of − 80 mV. Granisetron decreased the whole-cell inward conductance from 55 nS to 16 nS, while ondansetron decreased it from 55 nS to 34 nS. Insets show original current tracings recorded at − 140 mV; scale bars 2500 pA.

inward current at voltages more negative than − 90 mV (Figure 10). To quantify this effect, whole-cell conductance was measured between − 130 and − 100 mV, a range of potentials where membrane conductance is mostly determined by g_{K1}. Membrane conductance decreased from 45 ± 6 to 28 ± 4 nS ($n = 4$) with ondansetron, and from 40 ± 7 to 9 ± 2 nS ($n = 4$) with granisetron. The effect of ondansetron was almost completely reversible after 10 min washouts, while the effect of granisetron was only partially reversed after 20–30 min washouts. Granisetron was considerably more potent than ondansetron, as shown by the larger decrease of membrane conductance and by the important shift of the zero current potential to more depolarized potentials (from − 82 ± 2 mV to − 44 ± 8 mV).

Discussion

In cat ventricular myocytes, depolarizing voltage steps produced a small, gradually developing outward current that comprises the delayed rectifier (I_K) and other not yet identified conductances. Previous studies have shown that the outward currents obtained during the test potentials were variable in size and kinetic profile among myocytes (Spinelli *et al.*, 1993). Upon repolarization, each outward current trace was followed by a slowly decaying current tail. The tail currents were larger than the currents elicited during the depolarizing pulse, which is consistent with the high degree of inward rectification shown by I_K in cat ventricular myocytes (Follner *et al.*, 1992b). Tail currents increased with depolarization and reached full activation at positive potentials both after 250 and 2500 ms-long pulses. The longer pulses (2500 ms) activated larger tail currents at less positive potentials, which resulted in a 12 mV shift of the $V_{1/2}$ of the activation function. Nevertheless, maximum tail amplitude after 250 ms pulses was 90% of the maximum amplitude observed after 2500 ms pulses.

These results are consistent with the view that I_K in cat ventricular myocytes consists of a single, rapidly activating component similar to I_{Kr} of guinea-pig myocytes (Sanguinetti & Jurkiewicz, 1990). In agreement with this view, previous studies have shown the tail currents are abolished by WAY-123398 and E-4031, two specific blockers of I_K, even after very long pulses (5 s), whereas the time-dependent currents are only modestly affected even by very high drug concentrations (Follmer & Colatsky, 1990; Spinelli *et al.*, 1993). A single, rapidly activating, I_K has also been reported in rabbit ventricle (Carmeliet, 1992). Because of uncertainty on the nature of the conductances activated during the depolarizing pulse, and the difficulty of measuring small changes of current, in this study we decided to report only the results obtained with tail currents.

In voltage clamp studies, ondansetron and granisetron reversibly blocked I_K tail currents elicited after 2500 ms-long steps to + 35 mV with an estimated K_D of 1.7 ± 1.0 μM and 4.3 ± 1.7 μM, respectively. In current clamp experiments, ondansetron (1 μM) and granisetron (3 μM) prolonged APD_{90} by 30 ± 7% and 32 ± 8% without affecting other parameters of the transmembrane potential. Using a voltage clamp pro-

tocol that roughly approximated the voltage and duration of the plateau of an action potential (250 ms-long steps to + 15 mV), ondansetron and granisetron, at these same concentrations, blocked I_K tail currents by 41 ± 5% and 38 ± 4% without affecting the inward rectifier current. Thus, the class III effect observed in these studies is consistent with a block of the delayed rectifier current. At a significantly higher concentration (30 μM), both compounds blocked the inward rectifier. Granisetron was more potent than ondansetron and some blocking effect was evident at 10 μM, as indicated by a negative shift of holding current at − 45 mV.

Analysis of the activation curves permits a few conclusions regarding the mechanism of block of I_K. In the presence of the drug, the degree of block tracked the voltage-dependent activation of tail currents, suggesting that both 5-HT_3 antagonists blocked channels in the open state. Also, in the presence of the drug, the normalized activation curves were shifted in the hyperpolarized direction. The apparent negative voltage shift of the gating process may represent a voltage-dependent modulation of I_K that is distinct from voltage-dependence of block and is consistent with the hypothesis that the drug blocks the I_K channel in a given state (Hille, 1978; Carmeliet, 1993). Alternatively, the voltage shift may result from the block of a second component of I_K that is activated at more positive potentials. Finally, analysis of block at voltages where full activation has been attained revealed a modest increase of the amount of block with granisetron which is suggestive of an intrinsic voltage-dependent component of block.

Open-state block of the cardiac delayed rectifier has been reported with quinidine (Furukawa *et al.*, 1989), flecainide and encainide (Colatsky *et al.*, 1990; Follmer *et al.*, 1992a), dofetilide (Carmeliet, 1992), and almokalant (Carmeliet, 1993). Voltage-dependency of open-state block has been observed in a few studies. The block of the cloned human potassium channel HK2 by quinidine was voltage-dependent and was associated with an equivalent voltage-dependency of 0.19 (Snyders *et al.*, 1992). Block of the Shaker K^+ channel by tetraethylammonium (TEA) showed an equivalent voltage-dependency of 0.15 (Yellen *et al.*, 1991). Granisetron ($pK_a = 9.6$) is over 99% in the charged form at physiological pH. As for quinidine and TEA, our results are consistent with the hypothesis that the positively charged granisetron blocks the channel from the intracellular side of the membrane. In this case, the block by a positively charged molecule should increase in a voltage-dependent manner and δ, the equivalent voltage-dependency, can be interpreted to represent the fraction of the membrane electric field that the molecule must penetrate to reach the binding site. A similar effect was not observed with ondansetron. The reason for this difference is unclear. At physiological pH, ondansetron ($pK_a = 7.4$) is about 50% in the uncharged form. It is possible that the receptor for the charged molecule is outside the electric field of the membrane or that the block is due to the uncharged form. Due to the different proportion of the uncharged form, ondansetron is significantly more lipid soluble than granisetron. The octanol/buffer (pH = 7.4) partition coefficient for ondansetron is 1.70 compared to 0.73 for granisetron. Thus, ondansetron might also interact with the receptor via a hydrophobic pathway that is not available for the charged granisetron molecule. The higher lipophilicity of ondansetron might explain the faster and generally more complete washout observed with this molecule.

Changes in the time course of deactivation of the tail currents are also consistent with open channel block and unblock. If a significant fraction of channels are blocked and the rate of unblock is comparable to the rate of decay of the tail, the channels that become unblocked should transiently conduct outward current before closing and induce an apparent slowing of tail deactivation. This should result in a crossover with the control current, and, potentially, in a delay of the raising phase. The results obtained were consistent with this view, showing crossover of tail currents and a significant increase of τ with both ondansetron and granisetron. Significant delays of the peak current were observed, indicating that the unblock process affected the rate of raise of the tail current. This indicated that the rate of unblock was relatively slow, on the order of the rate of deactivation of the channels, but fast enough to induce a significant increase in outward current approximately within the first 20 ms. The observed changes in time course are similar to those reported for the block of the delayed rectifier by quaternary ammonium ions in the squid axon (Armstrong, 1971), by quinidine in rabbit (Furukawa *et al.*, 1989) and cloned human channels (Snyders *et al.*, 1992), and by class Ic antiarrhythmic drugs in cat ventricular myocytes (Follmer & Colatsky, 1990). Our results showed an appreciable contribution of open-state unblock to the tail current decay, but we cannot rule out the possibility that some trapping occurred in rested channels, as demonstrated with almokalant, a class III antiarrhythmic agent (Carmeliet, 1993).

Neuronal 5-HT_3 receptors are associated with an ion channel permeable to Na^+ and K^+ and showing a slightly higher selectivity for K^+ (Malone *et al.*, 1991). Thus, it is not surprising that 5-HT_3 antagonists can affect myocardial ion channels. Previous studies with various 5-HT_3 antagonists in whole animals have reported increases of ventricular refractoriness and prolongation of the QT interval of the ECG (Williams *et al.*, 1991), and some indication of antiarrhythmic effect (Williams *et al.*, 1985; Hof *et al.*, 1993). The data presented are consistent with open-state block and unblock of I_K by both ondansetron and granisetron. This profile of action should result in a use-dependent block of I_K. The amount of block after 250 and 2500 ms steps was similar, suggesting that a steady-state had been achieved after 250 ms and that, consequently, the τ of block should be less than 200 ms. The rates of unblock were not directly investigated. Fast block and slow unblock of I_K have been reported for quinidine and dofetilide (Furukawa *et al.*, 1989; Carmeliet, 1992). Such a profile is not considered favourable as steady-state block may be obtained at low rates of stimulation and no further increase of block would be obtained at faster rates (Carmeliet, 1993). The duration of the action potential is the resultant of several different conductances. Thus, it is not possible to translate directly changes at the level of the I_K channel in frequency-dependent effects on APD (Carmeliet, 1993; Spinelli *et al.*, 1993). In current clamp experiments, ondansetron behaved like dofetilide and quinidine in that the prolongation of APD was considerable at slow rates of stimulation and very limited at fast rates. A prolongation of APD characterized by reverse rate dependency could potentially carry the risk of proarrhythmic effects during a period of bradycardia. However, the concentrations required to block I_K (1–5 μM) are about 50 fold higher than the concentrations at which 5-HT_3 receptors are inhibited (Malone *et al.*, 1991), leaving an acceptable safety margin in therapy. Although metabolic phenotype and co-medication may affect this safety margin, present clinical experience with ondansetron does not show significant cardiovascular adverse effects (Markham & Sorkin, 1993).

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